

Notice of Allowability	Application No.	Applicant(s)	
	09/913,667	LIOTTA ET AL.	
	Examiner	Art Unit	
	N. M. Minnifield	1645	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☒ This communication is responsive to 11-15-04; 02-16-05; 3-1-05.
2. ☒ The allowed claim(s) is/are 1-28, 34-39, 44-51 and 53-87; now renumbered 1-77 respectively.
3. ☒ The drawings filed on 16 August 2001 are accepted by the Examiner.
4. ☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
 - a) ☒ All b) ☐ Some* c) ☐ None of the:
 1. ☐ Certified copies of the priority documents have been received.
 2. ☐ Certified copies of the priority documents have been received in Application No. _____.
 3. ☒ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

* Certified copies not received: _____.

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.

THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

5. ☐ A SUBSTITUTE OATH OR DECLARATION must be submitted. Note the attached EXAMINER'S AMENDMENT or NOTICE OF INFORMAL PATENT APPLICATION (PTO-152) which gives reason(s) why the oath or declaration is deficient.
 6. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
 - (a) ☐ including changes required by the Notice of Draftsperson's Patent Drawing Review (PTO-948) attached
 - 1) ☐ hereto or 2) ☐ to Paper No./Mail Date _____.
 - (b) ☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____.
- Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).**
7. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

- | | |
|---|--|
| 1. <input checked="" type="checkbox"/> Notice of References Cited (PTO-892) | 5. <input type="checkbox"/> Notice of Informal Patent Application (PTO-152) |
| 2. <input type="checkbox"/> Notice of Draftsperson's Patent Drawing Review (PTO-948) | 6. <input checked="" type="checkbox"/> Interview Summary (PTO-413),
Paper No./Mail Date <u>attached</u> . |
| 3. <input type="checkbox"/> Information Disclosure Statements (PTO-1449 or PTO/SB/08),
Paper No./Mail Date _____ | 7. <input checked="" type="checkbox"/> Examiner's Amendment/Comment |
| 4. <input type="checkbox"/> Examiner's Comment Regarding Requirement for Deposit
of Biological Material | 8. <input type="checkbox"/> Examiner's Statement of Reasons for Allowance |
| | 9. <input type="checkbox"/> Other _____. |

EXAMINER'S AMENDMENT

1. An examiner's amendment to the record appears below. Should the changes and/or additions be unacceptable to applicant, an amendment may be filed as provided by 37 CFR 1.312. To ensure consideration of such an amendment, it MUST be submitted no later than the payment of the issue fee.

Authorization for this examiner's amendment was given in a telephone interview with Anne Carlson, 47472/Tanya Harding, 42630 on February 16, 2005 and March 1, 2005

2. The application has been amended as follows:

1. (Currently amended) A method [of analyzing the protein content of a population of cells from a tissue sample,] comprising:

extracting [the] a population of about 1500 or fewer cells from [the] a tissue sample using microdissection under microscopic visualization;

isolating a protein sample from the extracted cell population, wherein isolating the protein sample comprises solubilizing contents of the extracted cell population in less than about 20 μ l of a buffer; and

analyzing the isolated protein sample.

2. (Currently amended) The method of claim 1 wherein [isolating the protein sample comprises solubilizing the extracted cell contents in less than about 20 μ l of a] the buffer [comprising] comprises at least one detergent to solubilize the cellular lipids, at least one proteinase inhibitor to preserve protein content and function, and at least one salt to lyse the nuclear contents.

3. (Previously presented) The method of claim 2 wherein the cell contents are solubilized in about 1 μ l to about 15 μ l of buffer.

4. (Previously presented) The method of claim 1 wherein analyzing the isolated protein sample comprises performing a soluble immunoassay using a labeled antibody specific for a protein of interest.

5. (Original) The method of claim 4 wherein the labeled antibody is labeled with a marker selected from the group consisting of colorimetric-detectable, chemiluminescence, fluorescence, and radioactivity.

6. (Currently amended) The method of claim 1[, wherein the method is a method of quantifying the amount of a protein of interest in a population of cells],

wherein extracting the population of cells from the tissue sample comprises laser capture microdissection; and

wherein isolating the protein sample from the extracted cell population comprises solubilizing the extracted cell contents in about 1 μ l to about 15 μ l of a buffer where the buffer comprises Tris-HCl, NONIDET[®] P40 (octylphenolpoly(ethyleneglycolether)[NP-40], sodium deoxycholate, sodium chloride, ethylenediaminetetraacetic acid [EDTA], aprotinin, leupeptin, sodium pyrophosphate, sodium orthovanadate, and 4-(2-aminoethyl)-bezenesulfonylfluoride [AEBSF]; and

wherein analyzing the isolated protein sample comprises performing a soluble immunoassay using an antibody specific for a protein of interest in the isolated protein sample, where the antibody is labeled with a marker selected from the group consisting of colorimetric-detectable, chemiluminescence, fluorescence, and radioactivity, and calibrating the assay to indicate the amount of the protein of interest present in the isolated protein sample.

7. (Previously presented) The method of claim 6 wherein the protein of interest in the isolated protein sample is prostate soluble antigen (PSA).

8. (Previously presented) The method of claim 1 wherein analyzing the isolated protein sample comprises:

performing a one dimensional polyacrylamide gel electrophoresis (1D PAGE) or two dimensional polyacrylamide gel electrophoresis (2D PAGE) to separate proteins in the isolated protein sample from each other; and

further analyzing the proteins in the isolated protein sample using a protein specific dye or Western blotting with a labeled antibody specific for the protein of interest in the isolated protein sample.

9. (Previously presented) The method of claim 1 wherein analyzing the isolated protein sample comprises

performing a two dimensional polyacrylamide gel electrophoresis (2D PAGE) to separate the proteins in the isolated protein sample from each other;

isolating a protein of interest from the gel; and

determining an amino acid sequence of the protein of interest.

10. (Original) The method of claim 9 wherein the sequence is determined using a method selected from the group consisting of N-terminal sequencing, mass spectrometry MS-MS sequencing, liquid chromatography quadrupole ion trap electrospray (LCQ-MS), and matrix assisted laser desorption/time of flight analysis (MALDI/TOF).

11. (Previously presented) The method of claim 1 wherein analyzing the isolated protein sample comprises performing surface enhanced laser desorption ionization spectroscopy (SELDI) to produce a protein fingerprint for the cell population.

12. (Original) The method of claim 1 wherein the cell population is microscopically identifiable as a tumor cell.

13. (Currently amended) The method of claim 1, [wherein the method is a method of characterizing binding properties of one or more intracellular proteins of a population of cells,] wherein analyzing the isolated protein sample comprises:

performing a one dimensional polyacrylamide gel electrophoresis (1D PAGE) or two dimensional polyacrylamide gel electrophoresis (2D PAGE) to separate the proteins from each other;

removing at least one protein of interest from the gel;

further analyzing the protein of interest by incubating the protein with a known or putative binding partner for the protein of interest; and

determining whether the protein of interest binds to the known or putative binding partner.

14. (Original) The method of claim 13 wherein the protein of interest is PSA and the known binding partner is alpha-1-antichymotrypsin (ACT).

15. (Currently amended) The method of claim 1, [wherein the method is a method of differentiating a protein content of at least two populations of cells of a tissue sample,] comprising:

extracting at least a first and a second population of cells directly from one or more tissue samples using laser capture microdissection;

isolating protein from the extracted cell populations to generate for each cell population an isolated protein sample having a content;

analyzing the isolated protein sample for at least two cell populations; and

comparing the protein content of the isolated protein sample of at least the first cell population to the protein content of the isolated protein sample of at least the second cell population to identify differing content.

16. (Currently amended) The method of claim 15 wherein [isolating protein comprises solubilizing the extracted cellular material in less than 20 μ l of a buffer wherein] the buffer comprises Tris-HCl, NONIDET[®] P40 (octylphenolpoly(ethyleneglycolether)), sodium deoxycholate, sodium chloride, ethylenediaminetetraacetic acid, aprotinin, leupeptin, sodium pyrophosphate, sodium orthovanadate, and 4-(2-aminoethyl)-benzenesulfonylfluoride.

17. (Previously presented) The method of claim 15 wherein the cell contents are solubilized in about 1 μ l to about 15 μ l of buffer.

18. (Previously presented) The method of claim 15 wherein analyzing the isolated protein comprises performing a soluble immunoassay using a labeled antibody specific for a protein of interest wherein the assay is calibrated to indicate the amount of the protein of interest present in the sample.

19. (Previously presented) The method of claim 18 wherein the immunoassay is of high sensitivity and the labeled antibody is labeled with a marker selected from the group consisting of colorimetric-detectable, chemiluminescence, fluorescence, and radioactive labels.

20. (Previously presented) The method of claim 15 wherein analyzing the isolated protein comprises:

performing a two dimensional polyacrylamide gel electrophoresis (2D PAGE) to separate proteins from each other;
isolating a protein of interest from the gel; and
determining an amino acid sequence of the protein of interest.

21. (Original) The method of claim 20 wherein the sequence is determined using a method selected from the group consisting of N-terminal sequencing, mass spectrometry MS-MS sequencing, liquid chromatography quadrupole ion trap electrospray (LCQ-MS), and matrix assisted laser desorption/time of flight analysis (MALDI/TOF).

22. (Previously presented) The method of claim 15 wherein analyzing the isolated protein comprises:

performing a one dimensional polyacrylamide gel electrophoresis (1D PAGE) or two dimensional polyacrylamide gel electrophoresis (2D PAGE) to separate protein fractions from each other; and

further analyzing the protein fractions using a protein specific dye or Western blotting with a labeled antibody specific for a protein of interest.

23. (Original) The method of claim 15 wherein the first population of cells and the second population of cells are from the same tissue sample and the first population is microscopically identifiable as tumor cells and the second population is microscopically identifiable as normal cells.

24. (Original) The method of claim 15 wherein the first population comprises several subpopulations wherein each subpopulation is microscopically identifiable as cells at different stages of tumor progression.

25. (Currently amended) The method of claim 1, [wherein the method is a method of comparing the protein content of a first population of cells microscopically identifiable as tumor cells to the protein content of a second population of cells that are normal wherein both populations of cells are extracted from the same tissue sample, the method] comprising:

extracting [the] first and second populations of about 1500 or fewer cells from the tissue sample using laser capture microdissection, in which a laser targets

the first and second populations as microscopically distinct and separates them from a larger microscopic structure; and

isolating a protein sample from each extracted cell population by solubilizing the extracted cell contents in about 1 μ l to about 15 μ l of a buffer where the buffer comprises Tris-HCl, NONIDET[®] P40 (octylphenolpoly(ethyleneglycolether)),, sodium deoxycholate, sodium chloride, ethylenediaminetetraacetic acid, aprotinin, leupeptin, sodium pyrophosphate, sodium orthovanadate, and 4-(2-aminoethyl)-benzenesulfonylfluoride; wherein analyzing each of the isolated protein samples comprises:

performing a one dimensional polyacrylamide gel electrophoresis or two dimensional polyacrylamide gel electrophoresis to separate proteins of the protein sample from each cell population;

further analyzing the separated proteins of each cell population using a protein specific dye or Western blotting with a labeled antibody specific for a protein of interest; and

comparing a protein of interest content of the first cell population to a protein of interest content of the second cell population.

26. (Currently amended) The method of claim 1, [wherein the method is a method of comparing the protein content of a first population of cells microscopically identifiable as tumor cells to the protein content of a second population of cells in order to identify the origin of the first population of cells, the method] comprising:

extracting [the] first and second populations of about 1500 or fewer cells from the tissue sample and from each other using laser capture microdissection;

isolating a protein sample from each extracted cell population by solubilizing cells from extracted cell populations in about 1 μ l to about 15 μ l of a buffer where the buffer comprises Tris-HCl, NONIDET[®] P40 (octylphenolpoly(ethyleneglycolether)), sodium deoxycholate, sodium chloride, ethylenediaminetetraacetic acid, aprotinin, leupeptin, sodium pyrophosphate, sodium orthovanadate, and 4-(2-aminoethyl)-benzenesulfonylfluoride; and wherein analyzing each of the isolated protein samples comprises:

performing surface enhanced laser desorption ionization spectroscopy (SELDI) to produce a protein fingerprint of the protein sample for each cell population; and

comparing the protein fingerprint of the first population of cells to the protein fingerprint of a known second population of cells to determine whether or not the two populations have the same origin.

27. (Currently amended) The method of claim 26 wherein [said] the first population of cells is microscopically identifiable as a tumor metastasis and the second population of cells is one of a battery of known normal tissue samples.

28. (Original) The method of claim 27 wherein the known normal tissue samples are from the same patient as the first population of cells.

29-33. (Cancelled)

34. (Currently amended) The method of claim 1, [wherein the method is a method of screening for the presence of a cellular component in a population of

cells from a tissue sample,] wherein isolating the protein sample from the extracted cell population comprises

lysing the extracted cell population to produce cellular components; and
wherein analyzing the isolated protein sample comprises:

immobilizing at least one cellular component or a binding agent in a
confined zone;

contacting the cellular components with a binding agent; and

detecting the interaction between the components and the binding
agent.

35. (Original) The method of claim 34 wherein the cellular component or the binding agent is labeled, and detecting the interaction between the cellular component and the binding agent comprises detecting the presence of the label.

36. (Previously presented) The method of claim 35 wherein the label is detected by a method selected from the group consisting of a colorimetric, chemiluminescent, radioactive, and fluorescence.

37. (Original) The method of claim 34 wherein the confined zone of the immobilized cellular component or the immobilized binding agent is an array.

38. (Original) The method of claim 34 wherein the cellular component is immobilized.

39. (Original) The method of claim 34 wherein the binding agent is immobilized.

40-43. (Cancelled).

44. (Previously presented) The method of claim 1, wherein analyzing the isolated protein sample comprises generating on a substrate an array comprising a series of at least two dilutions of the protein sample.

45. (Previously presented) The method of claim 44, wherein analyzing the isolated protein sample further comprises:
applying a first labeled probe that specifically detects a first protein analyte;
and
obtaining a quantitative value for the first protein analyte by comparing a signal from the first labeled probe at different positions in the dilution series.

46. (Previously presented) The method of claim 45, further comprising:
applying a second labeled probe that specifically detects a second protein analyte; and
obtaining a quantitative value for the second protein analyte by comparing a signal from the second labeled probe at different positions in the dilution series.

47. (Previously presented) The method of claim 6, wherein calibrating the assay comprises generating a serial dilution of the protein sample.

48. (Previously presented) The method of claim 15, wherein analyzing the isolated protein sample for at least two cell populations comprises generating on a substrate an array comprising a series of at least two dilutions of each protein sample.

49. (Previously presented) The method of claim 48, wherein analyzing the isolated protein sample for at least two cell populations further comprises:

applying a first labeled probe that specifically detects a first protein analyte;
and

obtaining a quantitative value for the first protein analyte by comparing a signal from the first labeled probe at different positions in each of the dilution series.

50. (Previously presented) The method of claim 49, further comprising:
applying a second labeled probe that specifically detects a second protein analyte; and

obtaining a quantitative value for the second protein analyte by comparing a signal from the second labeled probe at different positions in each of the dilution series.

51. (Previously presented) The method of claim 18, wherein calibrating the assay comprises generating a serial dilution of the protein sample.

52. (Cancelled).

53. (Previously presented) The method of claim 37 wherein the cellular component is immobilized.

54. (Previously presented) The method of claim 34, wherein the confined zone is a microspot on a microarray.

55. (Currently amended) The method of claim 1, wherein the isolated protein sample is referred to as a first isolated protein sample, [and the method] further [comprising] comprises [analyzing the protein content of at least a second population of cells from the tissue sample, or from a second tissue sample, which method comprises]:

extracting a second population of about 1500 or fewer cells from the tissue sample or [the] a second tissue sample;

isolating a second protein sample from the second extracted cell population;
and

analyzing the second isolated protein sample concurrently with the first isolated protein sample.

56. (Previously presented) The method of claim 55, wherein the protein contents of more than two populations of cells are analyzed.

57. (Previously presented) The method of claim 56, wherein the more than two populations of cells are extracted from more than two tissue samples.

58. (Previously presented) The method of claim 56, wherein the more than two populations of cells are extracted from:

tissues from different stages of malignancy;
tissues before and after a treatment;
tissues from different stages of development of an embryo; or
combinations thereof.

59. (Currently amended) A method [of analyzing the protein content of more than one population of cells from at least one tissue sample,] comprising:

extracting [the] more than one population of about 1500 or fewer cells from [the] at least one tissue sample(s) under microscopic visualization;

isolating a protein sample from each of the extracted cell populations, wherein isolating the protein sample comprises solubilizing contents of the extracted cell populations in less than about 20 μ l of a buffer; and

analyzing the isolated protein samples.

60. (Previously presented) The method of claim 59, wherein the cells are extracted from more than one tissue sample.

61. (Previously presented) The method of claim 60, wherein the more than one tissue samples are from a single subject.

62. (Previously presented) The method of claim 59, wherein extracting the more than one population of cells from the tissue samples comprises using microdissection.

63. (Previously presented) The method of claim 62, wherein the microdissection comprises laser capture microdissection.

64. (Previously presented) The method of claim 59, wherein the more than one population of cells extracted from the tissue sample(s) is cultured *in vitro* prior to the step of isolating the protein sample from each of the cell populations.

65. (Currently amended) The method of claim 64, wherein the more than [two] one populations of cells are extracted from:
tissues from different stages of malignancy;
tissues before and after a treatment;
tissues from different stages of development of an embryo; or
combinations thereof.

66. (Currently amended) The method of claim 59, [wherein the method is a method of screening for the presence of a cellular component in the more than one population of cells,] wherein isolating the protein sample from each of the extracted cell populations comprises:

lysing the extracted cell populations to produce cellular components;
and wherein analyzing the isolated protein sample from each of the extracted cell populations comprises:

immobilizing at least one cellular component or a binding agent in a confined zone;

contacting the cellular components with a binding agent; and

detecting the interaction between the components and the binding agent.

67. (Previously presented) The method of claim 66 wherein the cellular component is immobilized.

68. (Previously presented) The method of claim 67 wherein the confined zone of the immobilized cellular component or the immobilized binding agent is an array.

69. (Previously presented) The method of claim 66, wherein the confined zone is a microspot on a microarray.

70. (Previously presented) The method of claim 1 wherein analyzing the isolated protein comprises performing an immunoassay using a labeled antibody specific for a protein of interest, wherein the assay is calibrated to indicate the amount of the protein of interest present in the sample.

71. (Previously presented) The method of claim 70, wherein calibrating the assay comprises generating a serial dilution of the protein sample.

72. (Previously presented) The method of claim 34 wherein analyzing the isolated protein further comprises using a calibration to indicate the amount of the protein of interest present in the sample.

73. (Previously presented) The method of claim 72, wherein the calibration comprises generating a serial dilution of the protein sample.

74. (Currently amended) The method of claim 44, wherein analyzing the isolated protein sample further comprises generating on the substrate of the array a protein standard comprising a series of at least two dilutions of at least one purified protein.

75. (Previously presented) The method of claim 74, further comprising quantifying at least one protein in the protein sample, where the amount of protein is quantified in units relative to the amount of purified protein in the protein standard on the array.

76. (Previously presented) The method of claim 74, where the protein standard comprises a mixture of two or more purified proteins, and wherein each of the two or more purified proteins is used to calibrate quantification of at least one cellular component in at least one protein sample on the array.

77. (Previously presented) The method of claim 44, wherein each dilution is immobilized within a confined zone that can receive an individual reagent treatment.

78. (Previously presented) The method of claim 59, wherein analyzing the isolated protein sample comprises generating on a substrate an array comprising a series of at least two dilutions of the protein sample.

79. (Currently amended) The method of claim 78, wherein analyzing the isolated protein sample further comprises generating on the substrate of the array a protein standard comprising a series of at least two dilutions of at least one purified protein.

80. (Previously presented) The method of claim 79, further comprising quantifying at least one protein in the protein sample, where the amount of protein is quantified in units relative to the amount of purified protein in the protein standard on the array.

81. (Previously presented) The method of claim 79, where the protein standard comprises a mixture of two or more purified proteins, wherein each of the two or more purified proteins is used to calibrate quantification of at least one cellular component in at least one protein sample on the array.

82. (Previously presented) The method of claim 78, wherein each dilution is immobilized within a confined zone that can receive an individual reagent treatment.

83. (Currently amended) A method [of analyzing the protein content of a population of cells from a tissue sample by screening for the presence of a cellular component in a population of cells from a tissue sample,] comprising:

extracting [the] a population of about 1500 or fewer cells from [the] a tissue sample;

isolating a protein sample from the extracted cell population, wherein isolating the protein sample from the extracted cell population comprises lysing the extracted cell population in less than about 20 μ l of a buffer to produce cellular components; and

analyzing the isolated protein sample, wherein analyzing the isolated protein sample comprises:

generating on a substrate an array comprising a series of at least two dilutions of the protein sample;

contacting the array with a binding agent; and

detecting the interaction between the cellular components in the protein sample and the binding agent.

84. (Previously presented) The method of claim 1, wherein extracting the population of cells using microdissection under microscopic visualization comprises:

contacting the tissue sample with a transfer film;

focally activating the transfer film with a laser beam, thereby bonding the cells to the transfer film; and

lifting the bonded cells from the tissue sample, thereby extracting the population of cells and leaving unwanted cells behind.

85. (Currently amended) A method [of analyzing the protein content of a population of cells from a tissue sample,] comprising:

contacting [the] a tissue sample with a transfer film;

microscopically visualizing [the] a population of cells in the tissue sample;

focally activating the transfer film with a laser beam, thereby bonding the population of cells to the transfer film;

extracting the population of cells from the tissue sample, thereby leaving unwanted cells behind, to produce an extracted cell population of about 1500 or fewer cells;

isolating a protein sample from the extracted cell population, wherein isolating the protein sample comprises solubilizing contents of the extracted cell population in less than about 20 μ l of a buffer; and

analyzing the isolated protein sample.

86. (Currently amended) The method of claim 85, wherein [isolating the protein sample comprises solubilizing the extracted cell contents in less than about 20 μ l of a] the buffer [comprising] comprises at least one detergent to solubilize the cellular lipids, at least one proteinase inhibitor to preserve protein content and function, and at least one salt to lyse the nuclear contents.

87. (Previously presented) The method of claim 86 wherein the cell contents are solubilized in about 1 μ l to about 15 μ l of buffer.

3. The Liotta and Emmert-Buck Declarations under 37 CFR 1.132 filed November 15, 2004 is sufficient to overcome the rejection of claims 1-28, 34-39, 44-51 and 53-83 based upon 102(e).

4. Claims 1-28, 34-39, 44-51 and 53-87 have been allowed and now renumbered 1-77 respectively.

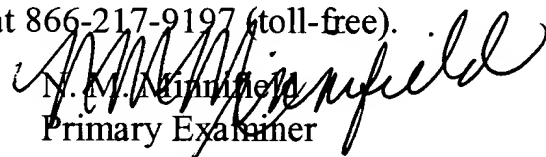
5. The following is an examiner's statement of reasons for allowance: The closest prior art does not disclose or suggest a method of extracting 1500 or fewer cells from a tissue sample using laser microdissection, isolating protein sample comprising solubilizing contents of the extracted cells using a 20 microliters or less of buffer and analyzing the isolated protein sample.

Any comments considered necessary by applicant must be submitted no later than the payment of the issue fee and, to avoid processing delays, should preferably accompany the issue fee. Such submissions should be clearly labeled "Comments on Statement of Reasons for Allowance."

6. Any inquiry concerning this communication or earlier communications from the examiner should be directed to N. M. Minnifield whose telephone number is 571-272-0860. The examiner can normally be reached on M-F (8:00-5:30) Second Friday Off.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Lynette R.F. Smith can be reached on 571-272-0864. The fax phone number for the organization where this application or proceeding is assigned is 703-872-9306.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).


N. M. A. Minfield
Primary Examiner
Art Unit 1645

NMM
March 2, 2005

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1. A method comprising:
extracting a population of about 1500 or fewer cells from a tissue sample
using microdissection under microscopic visualization;
isolating a protein sample from the extracted cell population, wherein
isolating the protein sample comprises solubilizing contents of the extracted cell
population in less than about 20 μ l of a buffer; and
analyzing the isolated protein sample.
2. The method of claim 1 wherein the buffer comprises at least one detergent to
solubilize the cellular lipids, at least one proteinase inhibitor to preserve protein
content and function, and at least one salt to lyse the nuclear contents.
3. The method of claim 2 wherein the cell contents are solubilized in about 1 μ l
to about 15 μ l of buffer.
4. The method of claim 1 wherein analyzing the isolated protein sample
comprises performing a soluble immunoassay using a labeled antibody specific for
a protein of interest.
5. The method of claim 4 wherein the labeled antibody is labeled with a marker
selected from the group consisting of colorimetric-detectable, chemiluminescence,
fluorescence, and radioactivity.

6. The method of claim 1,
wherein extracting the population of cells from the tissue sample comprises laser capture microdissection; and

wherein isolating the protein sample from the extracted cell population comprises solubilizing the extracted cell contents in about 1 μ l to about 15 μ l of a buffer where the buffer comprises Tris-HCl, NONIDET[®] P40

(octylphenolpoly(ethyleneglycolether)), sodium deoxycholate, sodium chloride, ethylenediaminetetraacetic acid, aprotinin, leupeptin, sodium pyrophosphate, sodium orthovanadate, and 4-(2-aminoethyl)-benzenesulfonylfluoride; and

wherein analyzing the isolated protein sample comprises performing a soluble immunoassay using an antibody specific for a protein of interest in the isolated protein sample, where the antibody is labeled with a marker selected from the group consisting of colorimetric-detectable, chemiluminescence, fluorescence, and radioactivity, and calibrating the assay to indicate the amount of the protein of interest present in the isolated protein sample.

7. The method of claim 6 wherein the protein of interest in the isolated protein sample is prostate soluble antigen (PSA).

8. The method of claim 1 wherein analyzing the isolated protein sample comprises:

performing a one dimensional polyacrylamide gel electrophoresis (1D PAGE) or two dimensional polyacrylamide gel electrophoresis (2D PAGE) to separate proteins in the isolated protein sample from each other; and

further analyzing the proteins in the isolated protein sample using a protein specific dye or Western blotting with a labeled antibody specific for the protein of interest in the isolated protein sample.

9. The method of claim 1 wherein analyzing the isolated protein sample comprises

performing a two dimensional polyacrylamide gel electrophoresis (2D PAGE) to separate the proteins in the isolated protein sample from each other; isolating a protein of interest from the gel; and determining an amino acid sequence of the protein of interest.

10. The method of claim 9 wherein the sequence is determined using a method selected from the group consisting of N-terminal sequencing, mass spectrometry MS-MS sequencing, liquid chromatography quadrupole ion trap electrospray (LCQ-MS), and matrix assisted laser desorption/time of flight analysis (MALDI/TOF).

11. The method of claim 1 wherein analyzing the isolated protein sample comprises performing surface enhanced laser desorption ionization spectroscopy (SELDI) to produce a protein fingerprint for the cell population.

12. The method of claim 1 wherein the cell population is microscopically identifiable as a tumor cell.

13. The method of claim 1, wherein analyzing the isolated protein sample comprises:

- performing a one dimensional polyacrylamide gel electrophoresis (1D PAGE) or two dimensional polyacrylamide gel electrophoresis (2D PAGE) to separate the proteins from each other;

- removing at least one protein of interest from the gel;

- further analyzing the protein of interest by incubating the protein with a known or putative binding partner for the protein of interest; and

- determining whether the protein of interest binds to the known or putative binding partner.

14. The method of claim 13 wherein the protein of interest is PSA and the known binding partner is alpha-1-antichymotrypsin (ACT).

15. The method of claim 1, comprising:

- extracting at least a first and a second population of cells directly from one or more tissue samples using laser capture microdissection;

- isolating protein from the extracted cell populations to generate for each cell population an isolated protein sample having a content;

- analyzing the isolated protein sample for at least two cell populations; and

- comparing the protein content of the isolated protein sample of at least the first cell population to the protein content of the isolated protein sample of at least the second cell population to identify differing content.

16. The method of claim 15 wherein the buffer comprises Tris-HCl, NONIDET[®] P40 (octylphenolpoly(ethyleneglycolether)), sodium deoxycholate, sodium chloride, ethylenediaminetetraacetic acid, aprotinin, leupeptin, sodium pyrophosphate, sodium orthovanadate, and 4-(2-aminoethyl)-benzenesulfonylfluoride.
17. The method of claim 15 wherein the cell contents are solubilized in about 1 μ l to about 15 μ l of buffer.
18. The method of claim 15 wherein analyzing the isolated protein comprises performing a soluble immunoassay using a labeled antibody specific for a protein of interest wherein the assay is calibrated to indicate the amount of the protein of interest present in the sample.
19. The method of claim 18 wherein the immunoassay is of high sensitivity and the labeled antibody is labeled with a marker selected from the group consisting of colorimetric-detectable, chemiluminescence, fluorescence, and radioactive labels.
20. The method of claim 15 wherein analyzing the isolated protein comprises:
 - performing a two dimensional polyacrylamide gel electrophoresis (2D PAGE) to separate proteins from each other;
 - isolating a protein of interest from the gel; and
 - determining an amino acid sequence of the protein of interest.

21. The method of claim 20 wherein the sequence is determined using a method selected from the group consisting of N-terminal sequencing, mass spectrometry MS-MS sequencing, liquid chromatography quadrupole ion trap electrospray (LCQ-MS), and matrix assisted laser desorption/time of flight analysis (MALDI/TOF).

22. The method of claim 15 wherein analyzing the isolated protein comprises:
performing a one dimensional polyacrylamide gel electrophoresis (1D PAGE) or two dimensional polyacrylamide gel electrophoresis (2D PAGE) to separate protein fractions from each other; and
further analyzing the protein fractions using a protein specific dye or Western blotting with a labeled antibody specific for a protein of interest.

23. The method of claim 15 wherein the first population of cells and the second population of cells are from the same tissue sample and the first population is microscopically identifiable as tumor cells and the second population is microscopically identifiable as normal cells.

24. The method of claim 15 wherein the first population comprises several subpopulations wherein each subpopulation is microscopically identifiable as cells at different stages of tumor progression.

25. The method of claim 1, comprising:
extracting first and second populations of about 1500 or fewer cells from the tissue sample using laser capture microdissection, in which a laser targets the first

and second populations as microscopically distinct and separates them from a larger microscopic structure; and

isolating a protein sample from each extracted cell population by solubilizing the extracted cell contents in about 1 μ l to about 15 μ l of a buffer where the buffer comprises Tris-HCl, NONIDET[®] P40 (octylphenolpoly(ethyleneglycolether)), sodium deoxycholate, sodium chloride, ethylenediaminetetraacetic acid, aprotinin, leupeptin, sodium pyrophosphate, sodium orthovanadate, and 4-(2-aminoethyl)-benzenesulfonylfluoride; wherein analyzing each of the isolated protein samples comprises:

performing a one dimensional polyacrylamide gel electrophoresis or two dimensional polyacrylamide gel electrophoresis to separate proteins of the protein sample from each cell population;

further analyzing the separated proteins of each cell population using a protein specific dye or Western blotting with a labeled antibody specific for a protein of interest; and

comparing a protein of interest content of the first cell population to a protein of interest content of the second cell population.

26. The method of claim 1, comprising:

extracting first and second populations of about 1500 or fewer cells from the tissue sample and from each other using laser capture microdissection;

isolating a protein sample from each extracted cell population by solubilizing cells from extracted cell populations in about 1 μ l to about 15 μ l of a buffer where the buffer comprises Tris-HCl, NONIDET[®] P40 (octylphenolpoly(ethyleneglycolether)), sodium deoxycholate, sodium chloride,

ethylenediaminetetraacetic acid, aprotinin, leupeptin, sodium pyrophosphate, sodium orthovanadate, and 4-(2-aminoethyl)-benzenesulfonylfluoride; and wherein analyzing each of the isolated protein samples comprises:

performing surface enhanced laser desorption ionization spectroscopy (SELDI) to produce a protein fingerprint of the protein sample for each cell population; and

comparing the protein fingerprint of the first population of cells to the protein fingerprint of a known second population of cells to determine whether or not the two populations have the same origin.

27. The method of claim 26 wherein the first population of cells is microscopically identifiable as a tumor metastasis and the second population of cells is one of a battery of known normal tissue samples.

28. The method of claim 27 wherein the known normal tissue samples are from the same patient as the first population of cells.

34. The method of claim 1, wherein isolating the protein sample from the extracted cell population comprises

lysing the extracted cell population to produce cellular components; and wherein analyzing the isolated protein sample comprises:

immobilizing at least one cellular component or a binding agent in a confined zone;

contacting the cellular components with a binding agent; and

detecting the interaction between the components and the binding agent.

35. The method of claim 34 wherein the cellular component or the binding agent is labeled, and detecting the interaction between the cellular component and the binding agent comprises detecting the presence of the label.

36. The method of claim 35 wherein the label is detected by a method selected from the group consisting of a colorimetric, chemiluminescent, radioactive, and fluorescence.

37. The method of claim 34 wherein the confined zone of the immobilized cellular component or the immobilized binding agent is an array.

38. The method of claim 34 wherein the cellular component is immobilized.

39. The method of claim 34 wherein the binding agent is immobilized.

44. The method of claim 1, wherein analyzing the isolated protein sample comprises generating on a substrate an array comprising a series of at least two dilutions of the protein sample.

45. The method of claim 44, wherein analyzing the isolated protein sample further comprises:

applying a first labeled probe that specifically detects a first protein analyte;
and

obtaining a quantitative value for the first protein analyte by comparing a
signal from the first labeled probe at different positions in the dilution series.

46. The method of claim 45, further comprising:

applying a second labeled probe that specifically detects a second protein
analyte; and

obtaining a quantitative value for the second protein analyte by comparing a
signal from the second labeled probe at different positions in the dilution series.

47. The method of claim 6, wherein calibrating the assay comprises generating a
serial dilution of the protein sample.

48. The method of claim 15, wherein analyzing the isolated protein sample for at
least two cell populations comprises generating on a substrate an array comprising
a series of at least two dilutions of each protein sample.

49. The method of claim 48, wherein analyzing the isolated protein sample for at
least two cell populations further comprises:

applying a first labeled probe that specifically detects a first protein analyte;
and

obtaining a quantitative value for the first protein analyte by comparing a
signal from the first labeled probe at different positions in each of the dilution
series.

50. The method of claim 49, further comprising:
applying a second labeled probe that specifically detects a second protein analyte; and
obtaining a quantitative value for the second protein analyte by comparing a signal from the second labeled probe at different positions in each of the dilution series.
51. The method of claim 18, wherein calibrating the assay comprises generating a serial dilution of the protein sample.
53. The method of claim 37 wherein the cellular component is immobilized.
54. The method of claim 34, wherein the confined zone is a microspot on a microarray.
55. The method of claim 1, wherein the isolated protein sample is referred to as a first isolated protein sample, and the method further comprises:
extracting a second population of about 1500 or fewer cells from the tissue sample or a second tissue sample;
isolating a second protein sample from the second extracted cell population;
and
analyzing the second isolated protein sample concurrently with the first isolated protein sample.

56. The method of claim 55, wherein the protein contents of more than two populations of cells are analyzed.

57. The method of claim 56, wherein the more than two populations of cells are extracted from more than two tissue samples.

58. The method of claim 56, wherein the more than two populations of cells are extracted from:

- tissues from different stages of malignancy;
- tissues before and after a treatment;
- tissues from different stages of development of an embryo; or
- combinations thereof.

59. A method comprising:

- extracting more than one population of about 1500 or fewer cells from at least one tissue sample(s) under microscopic visualization;

- isolating a protein sample from each of the extracted cell populations, wherein isolating the protein sample comprises solubilizing contents of the extracted cell populations in less than about 20 μ l of a buffer; and
- analyzing the isolated protein samples.

60. The method of claim 59, wherein the cells are extracted from more than one tissue sample.

61. The method of claim 60, wherein the more than one tissue samples are from a single subject.

62. The method of claim 59, wherein extracting the more than one population of cells from the tissue samples comprises using microdissection.

63. The method of claim 62, wherein the microdissection comprises laser capture microdissection.

64. The method of claim 59, wherein the more than one population of cells extracted from the tissue sample(s) is cultured *in vitro* prior to the step of isolating the protein sample from each of the cell populations.

65. The method of claim 64, wherein the more than one populations of cells are extracted from:

tissues from different stages of malignancy;

tissues before and after a treatment;

tissues from different stages of development of an embryo; or

combinations thereof.

66. The method of claim 59, wherein isolating the protein sample from each of the extracted cell populations comprises:

lysing the extracted cell populations to produce cellular components;

and wherein analyzing the isolated protein sample from each of the extracted cell populations comprises:

immobilizing at least one cellular component or a binding agent in a confined zone;

contacting the cellular components with a binding agent; and

detecting the interaction between the components and the binding agent.

67. The method of claim 66 wherein the cellular component is immobilized.

68. The method of claim 67 wherein the confined zone of the immobilized cellular component or the immobilized binding agent is an array.

69. The method of claim 66, wherein the confined zone is a microspot on a microarray.

70. The method of claim 1 wherein analyzing the isolated protein comprises performing an immunoassay using a labeled antibody specific for a protein of interest, wherein the assay is calibrated to indicate the amount of the protein of interest present in the sample.

71. The method of claim 70, wherein calibrating the assay comprises generating a serial dilution of the protein sample.

72. The method of claim 34 wherein analyzing the isolated protein further comprises using a calibration to indicate the amount of the protein of interest present in the sample.

73. The method of claim 72, wherein the calibration comprises generating a serial dilution of the protein sample.

74. The method of claim 44, wherein analyzing the isolated protein sample further comprises generating on the substrate of the array a protein standard comprising a series of at least two dilutions of at least one purified protein.

75. The method of claim 74, further comprising quantifying at least one protein in the protein sample, where the amount of protein is quantified in units relative to the amount of purified protein in the protein standard on the array.

76. The method of claim 74, where the protein standard comprises a mixture of two or more purified proteins, and wherein each of the two or more purified proteins is used to calibrate quantification of at least one cellular component in at least one protein sample on the array.

77. The method of claim 44, wherein each dilution is immobilized within a confined zone that can receive an individual reagent treatment.

78. The method of claim 59, wherein analyzing the isolated protein sample comprises generating on a substrate an array comprising a series of at least two dilutions of the protein sample.

79. The method of claim 78, wherein analyzing the isolated protein sample further comprises generating on the substrate of the array a protein standard comprising a series of at least two dilutions of at least one purified protein.

80. The method of claim 79, further comprising quantifying at least one protein in the protein sample, where the amount of protein is quantified in units relative to the amount of purified protein in the protein standard on the array.

81. The method of claim 79, where the protein standard comprises a mixture of two or more purified proteins, wherein each of the two or more purified proteins is used to calibrate quantification of at least one cellular component in at least one protein sample on the array.

82. The method of claim 78, wherein each dilution is immobilized within a confined zone that can receive an individual reagent treatment.

83. A method comprising:
extracting a population of about 1500 or fewer cells from a tissue sample;
isolating a protein sample from the extracted cell population, wherein
isolating the protein sample from the extracted cell population comprises lysing the
extracted cell population in less than about 20 μ l of a buffer to produce cellular
components; and
analyzing the isolated protein sample, wherein analyzing the isolated protein
sample comprises:

generating on a substrate an array comprising a series of at least two dilutions of the protein sample;
contacting the array with a binding agent; and
detecting the interaction between the cellular components in the protein sample and the binding agent.

84. The method of claim 1, wherein extracting the population of cells using microdissection under microscopic visualization comprises:

contacting the tissue sample with a transfer film;
focally activating the transfer film with a laser beam, thereby bonding the cells to the transfer film; and
lifting the bonded cells from the tissue sample, thereby extracting the population of cells and leaving unwanted cells behind.

85. A method comprising:

contacting a tissue sample with a transfer film;
microscopically visualizing a population of cells in the tissue sample;
focally activating the transfer film with a laser beam, thereby bonding the population of cells to the transfer film;
extracting the population of cells from the tissue sample, thereby leaving unwanted cells behind, to produce an extracted cell population of about 1500 or fewer cells;
isolating a protein sample from the extracted cell population, wherein isolating the protein sample comprises solubilizing contents of the extracted cell population in less than about 20 μ l of a buffer; and

analyzing the isolated protein sample.

86. The method of claim 85, wherein the buffer comprising at least one detergent to solubilize the cellular lipids, at least one proteinase inhibitor to preserve protein content and function, and at least one salt to lyse the nuclear contents.

87. The method of claim 86 wherein the cell contents are solubilized in about 1 μ l to about 15 μ l of buffer.